Cytochrome spectra of cytoplasmic mutants

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Abstract
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2) Filtration survivors were plated onto arginine plus proline by me but onto ornithine by Davis. Several vigorous colonies selected by this procedure after maturation with MNNG were crossed to wild type. Among the prototrophic progeny were several which had no detectable arginase on minimal or on minimal plus arginine. (Wild type grown on "N" has a specific activity of about 2 μmoles ornithine/mgm protein/minute, which is increased some two-fold on 3 mM arginine. The assay procedure would easily detect 0.1% of wild type activity.)

The uptake of arginine by one arginase mutant (aga-2.2) has been used in studying C14-arginine. Although the data do not permit the calculation of precise Km values for wild type and mutant strains, it is clear that there is no gross disturbance of arginine uptake in these mutants. As expected, however, the mutants will not utilize arginine as sole nitrogen source. Like those isolated by Davis, the mutants are strongly inhibited by arginine. The mean dry weight of triplicate 26-hour shake flask pods of aga-2.2 was reduced by 41% in 0.1 mM arginine, by 73% in 1.0 mM arginine and 90% by 3 mM arginine.

A further point of interest is that a five-fold induction of ornithine transaminase (OT) by arginine has been demonstrated in aga-2.2 (although no induction was observed in another aga mutant in a parallel experiment.) The induction of OT by arginine in the absence of arginase would preclude a sequential mode of induction via ornithine and would support the idea that arginase and ornithine transaminase function together in arginine catabolism.

One enzyme of arginine biosynthesis, acetylornithine transaminase, has been assayed in aga-2 grown in minimal with STA4 as a wild type control. Specific activities of 22-hour shake flask pods, 17.3 μmole/mg/min for STA4 and 14.4 μmole/mg/min for aga-2.2, were not significantly different, in view of the variation between cultures normally encountered in this assay.

This work was supported by the Gosney Fund, California Institute of Technology. The hospitality and encouragement of Professor N. H. Horowitz was greatly appreciated. — — John Innes Institute, Colney Lane, Norwich NR7 OF, England.

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Cytochrome spectra of three cytoplasmic mutants.

The cytochrome difference spectra obtained from mitochondria from several cytoplasmic mutants of Neurospora were described by Griffiths et al. (Neurospora NewsL. 13: 16). The list of the mutants that were examined did not include [SG-1], [exn-1] and [step-c]. A culture of [SG-1] (RL3202-23), which presumably has cytoplasmic continuity with the acriflavine-induced [SG] (Srb 1958 Cold Spring Harbor Symp. Quant. Biol. 23: 269), was kindly provided by A. M. Srb. The other two mutants, [exn-1] and [step-c], were obtained after N-methyl-N'-nitro-N-nitroso-guanidine treatments of conidia and vegetatively growing cultures (Bertrand and Pittenger 1968 Genetics 60: 161). Mitochondria from young cultures of these three mutants contain the three major cytochromes in relative amounts comparable to mitochondria from the group that includes [pox]. One of the distinctive features in the absence of cytochromes a + aq (600 μm), low amounts of cytochrome b (560 μm) and on excess of cytochrome c (550 μm). [SG-1] differs from the other mutants in that its difference spectrum has an exceptionally pronounced R-peak for cytochrome c. Whether this feature is characteristic of [SG-1] or is specific to strain RL3202-23 has not yet been determined. — — Division of Biology, Kansas State University, Manhattan, Kansas 66502.

The treated conidio were overplated an petri dishes of sorbose medium at a concentration of 1-2 x 10⁶ conidio per petri dish. Eighteen hours after plating, a third layer containing lysine and canavanine was added to the petri dishes to reduce the residual leaky growth of the unmutated aga-2 conidia. All steps involving ICR-170 were carried out in red light to prevent the occurrence of photodynamic mutation, and as an added precaution the plates were kept in darkness or red light for 24 hours after treatment. The plates were scored after incubation at 25°C for seven days.

Compared to the data on the related compound acridine yellow (Reissig 1964 Neurospora NewsL. 6: 16), there is no doubt that ICR-170 is an effective mutagen. The differences between the concentration-dependent curve (figure la) in which mutation rate and kill increase linearly with dose, and the time-dependent curve (figure lb), show that ICR-170 is very rapidly taken up into the conidio, and has its maximum effect within the first few minutes of exposure. The mutants induced in this experiment are currently being investigated to determine their nature. This work was supported by NIH Grant No. AI-01462. — — Department of Biological Sciences, Stanford University, Stanford, California 94305.